The Colours of Pollen Available to Honey Bees through the Year

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Introduction

The high rate of colony loss in Europe and the USA has prompted considerable research and speculation as to the possible causes (Neumann & Carreck, 2010; van der Zee et al., 2014; Kulhanek et al., 2017). One contributory factor could be poor bee nutrition resulting from habitat loss and changes in land use that have reduced the quality and diversity of available pollen (Naug, 2009; Brodschneider & Crailsheim, 2010; Höcherl, Siede, Illies, Gaetschenberger & Tautz, 2012; Odoux et al., 2012; Di Pasquale et al., 2013; Williams & Carreck, 2014). As a result there has been an increased interest in research on pollen availability and pollen collection by honey bees.

In many countries, the plants from which honey bees collect pollen have been surveyed and are well known (e.g. Kirk, 2006; Kirk & Howes, 2012), but there have been very few systematic or long-term studies of the species of pollen collected by individual honey bee colonies, and these studies have usually lasted no more than two or three years, so there are few data that can be used to assess pollen quality or diversity for honey bees and test whether this has declined over decades. The few quantitative studies in the United Kingdom have recorded totals of 48-100 species of pollen at a site over one or two seasons and more species were collected in urban sites than rural sites (Percival, 1947; Synge, 1947; Thomas, 1983). More recent studies include an agrarian environment in western France (Odoux et al., 2012) and mistbelt forests in South Africa (Mensah, Veldtman & Seifert, 2017).

An international project is currently measuring the diversity of pollen collected by honey bees in Europe and part of this involves asking beekeepers to count the number of different pollen colours in samples from pollen traps on their hives (Van der Steen & Brodschneider, 2014). Pollen-load colours can be used to record the diversity of plants from which bees forage (Kirk, 2006), but caution is needed because one plant species can produce more than one pollen colour and very different species can have pollen loads with identical colours (Hodges, 1952; Hidalgo & Bootello, 1990; Kirk, 2006). However, measurements have shown that there is a good correlation between colour analysis and palynological analysis (Conti et al., 2016).

In this article, I have not analysed the pollens collected by individual honey bee colonies. Instead, I have analysed the patterns in the production of pollen by the plants from which honey bees are known to collect pollen. This is pollen production from the botanical

perspective. However, it can serve as a null hypothesis of what we would expect if honey bee pollens were randomly sampled by honey bees. Would we expect to see similar pollen colours throughout the season or are there seasonal patterns? There is evidence that the predominant pollen-load colours can change predictably through the season. For example, Hodges (1952) observed in southern England that 'green' was common from March to May and that during the 'green period' it was sometimes impossible to separate different plant sources by colour. Pollen loads collected from pollen traps on hives at Keele University in central England in the 1990s also showed a predominance of yellow-green pollen loads in the first half of the season, followed by an increase in the proportion of orange. These patterns were not simply the result of one or two dominant species.

I have analysed 943 colour records for pollen loads collected by honey bees from 307 plant species (313 distinct taxa) in England and Germany. These were collected from bees foraging at known plants and checked microscopically, so the identity of the source plant is known. They include wild species, garden plants and crops from 65 families.

I have tested for patterns in the pollens available through the season. Do the major plant families have characteristic pollen-load colours or times of flowering? If so, do the colours collected by honey bees through the season reflect these patterns? Does the diversity of available pollen-load colours change through the season?

The analysis has focused on the families Asteraceae (e.g. dandelion, sunflower, thistle, knapweed) and Rosaceae (e.g. apple, cherry, hawthorn, blackberry). These families are large and had the most species in the data set. They contain many species that are major forage sources for honey bees, and in a survey of over 400 honey samples across England and Wales, most species of pollen were from these two families (Roberts, 2014).

It must be emphasised that the data presented here do not show what individual colonies actually collect. In any one area, only some of the species will be present and some species will be much more abundant than others. However, the data show underlying patterns in the pool of what is potentially available that can be used to interpret data for pollen loads collected by individual honey bee colonies.

Materials and Methods

Recording pollen-load colours

The colours of pollen loads collected by honey bees (*Apis mellifera* L.) were recorded by taking pollen loads from the legs of individual live honey bees and matching them by eye to comprehensive printed colour charts in bright, indirect (north) daylight. The method is described in detail elsewhere (Kirk, 1994). The records combine those of Dorothy Hodges in England from 1946-1951 and those of William Kirk in England from 1990-1999 and in Germany from 1993-1997, and nearly all of the colour records have been published previously in the form of colour identification guides to pollen loads (Hodges, 1952; Kirk, 2006). The term ‘colour’ is used here in the broad sense to refer to a particular combination of hue, lightness and saturation in the range visible to the human eye (Wright, 1969).

The data included multiple records for most species. To simplify the data for analysis and presentation, all the colours were divided into six categories: (1) red; (2) brown; (3) orange; (4) yellow-green (including yellow and green); (5) pale (including white); and (6) purple-blue (including grey and black). The categorisation was done by eye and assisted by the arrangement of the colours in systematic colour charts. It was not practical to separate yellow and green because of the many yellow-green intermediates. The categories were chosen because, in practice, they provided reasonably distinct categories into which pollen loads could be separated easily. This categorisation has been used successfully to present colours in a way that is easy to use for beekeepers (Kirk, 2006). The data were then reduced to one colour category record for each plant species. Nearly all species had all their records in the same colour category, but six species had forms or cultivars in two different colour categories and these were then recorded in both colour categories. The data for analysis included 313 taxa (species, forms or cultivars) from 307 plant species in 65 families.

Plant species and flowering times

The pollen loads were recorded extensively in England (1946-1951 and 1990-1999) and Germany (1993-1997) and included wild species, garden plants and crops, thus including the full range of plant species that honey bees encounter in rural and urban areas.

Plant species and families followed the classification of Stace (2010). Months of flowering were based on personal experience and data from published flora (Clapham, Tutin & Moore, 1987). Plant species that flower in more than one month were included in the counts for each month in which they are in flower.

Data analysis

The contingency tables were analysed with chi-squared tests using IBM SPSS Statistics version 21 (IBM Corporation, USA). Since some cells in the tables contained sparse data, the *P*-values were obtained by the Monte Carlo method in the SPSS Exact Tests module, using sufficient samples to give a *P*-value accurate to three decimal places with 99% confidence. Months or colour categories that included no taxa were omitted from the relevant test.

Simpson's diversity index (*D*) (Begon, Townsend & Harper, 2006) in the form

$$D = \frac{1}{\sum_{i=1}^{S} p_i^2}$$

was used as a measure of pollen colour diversity for the taxa in flower each month. It was selected because it is a simple and intuitive measure that reflects both the number of colours and their equitability (evenness). The index is commonly used for species diversity, but in this case S is the number of colour categories ($S = 6$) and p is the proportion of taxa in flower in each colour category in that month. Since there are six colour categories, the index could theoretically range from $D = 1$, if all the taxa were in one colour category, to $D = 6$, if all six colour categories were present and represented by equal numbers of taxa. The index can be considered as analogous to the number of colour categories likely to be obtained in a small, random sample of taxa.

Results

The number of taxa in flower peaked in summer (July) and was lowest in late autumn and winter (November to January) (Fig. 1A). [Fig. 1 near here] This was unsurprising, but the two largest families in the data set did not follow the same pattern. Although at least some taxa were in flower in most months, Rosaceae ($n = 39$) peaked earlier, in June, whereas Asteraceae ($n = 51$) peaked later, in August (Fig. 1B). The taxa in all the other families

combined (n = 223) peaked between them in July. A comparison of the frequencies of taxa in flower each month showed a highly significant difference between Rosaceae and Asteraceae ($X^2_{(8)} = 87.8$, $P < 0.001$).

The most frequent colours from the 313 taxa were yellow-green (34%) and orange (30%) (Table 1). [Table 1 near here] The predominant pollen colours changed through the season. Yellow-green peaked in June, orange peaked in July and pale peaked in August (Fig. 1C). Rosaceae and Asteraceae included a range of pollen colours, but Rosaceae included mainly yellow-green and brown, whereas Asteraceae included mainly orange and pale (Table 1). Within the Asteraceae, orange pollens were predominant in the sub-family Asteroideae (e.g. sunflower), whereas pale pollens were predominant in the sub-family Carduoideae (e.g. knapweed), demonstrating further phylogenetic association. The frequencies of taxa in each colour category were significantly different between Rosaceae and Asteraceae ($X^2_{(3)} = 57.8$, $P < 0.001$), between Rosaceae and other families ($X^2_{(5)} = 21.2$, $P = 0.001$) and between Asteraceae and other families ($X^2_{(5)} = 52.7$, $P < 0.001$) (Table 1).

Considering flowering time and pollen colour together, Rosaceae flowered earlier and was predominantly yellow-green and brown, whereas Asteraceae flowered later and was predominantly orange and pale. While this apparent phylogenetic constraint on flowering time and pollen colour contributes to the overall pattern of early yellow-green and late orange and pale (Fig. 1C), it does not explain it completely. Rosaceae and Asteraceae only account for 12% and 16% of the taxa respectively and without them the same colour sequence through the season was still evident, although weaker.

Pollen colour diversity, using Simpson's index, increased through the season, peaking in September and then dropping off rapidly to a minimum in December (Fig. 1D).

Discussion

The data showed a clear seasonal pattern in the colours of available pollen and this matched the seasonal pattern of pollen colours collected by honey bee colonies that was previously observed by Hodges (1952) in southern England and also observed at Keele University in central England (see Introduction). Evidence is presented that this is a consequence, at least partly, of a phylogenetic association between flowering time and pollen colour. This was shown for the two largest families, Rosaceae and Asteraceae, but the seasonal pattern of

colour was still present when these families were excluded from the data. The pattern in other families may result from wider phylogenetic affinities (at the level of order or class) or possibly from some evolutionary advantage in different pollen colours through the season. For example, pollen pigments may protect the genetic content of the pollen from light radiation (Stanley, 1971), and the advantage of this is likely to differ according to flowering period and whether pollen is exposed in flowers. Another possibility is that the known association between flower colour and pollinator class (Faegri & van der Pijl, 1979) may extend to pollen colour, which is often but not always similar to flower colour, and so pollen colour may have adapted to the relative availability of different pollinator classes through the season.

The phylogenetic association between flowering time and pollen colour will reduce the phylogenetic diversity and colour diversity of pollen available at one time. However, honey bees do not forage randomly, but tend to forage from the main sources available at the time (Percival, 1947; Visscher & Seeley, 1982). While this behaviour is efficient for foraging, it will also reduce the diversity of pollen collection compared to the pool of what is available. The combination of phylogenetic constraint and selective foraging behaviour means that measures of pollen diversity, such as colour diversity, over very short periods (days) may under-estimate the diversity of pollen collected by a colony and measures over longer periods (weeks or months) could be much higher.

Can these findings be generalized beyond England and Germany where the data were collected? The records were for wild species, garden plants and crops, and most also occur in surrounding countries with similar climates, so the findings may be regionally applicable. In addition, the two main families in the study, Rosaceae and Asteraceae, are large families found worldwide, so the phylogenetic constraints on pollen colour and flowering time identified in these families may well be applicable more generally.

During the data collection, I spent many hours searching botanic gardens for honey bees with pollen loads. Despite the high diversity of plants in flower, it was not uncommon to find large numbers of honey bees on a large flowering tree, such as a willow (*Salix* species) and at the same time none on many other plants that were equally suitable for honey bee foraging but were not presenting so many flowers. The bees appeared to be prioritising efficiency of pollen collection (quantity) over collecting pollen from many plant species (diversity). This was also observed in the National Botanic Garden of Wales, where pollen in honey came

from only 11% of the 437 available genera (de Vere et al., 2017), as well as in mistbelt forests in South Africa where only a few of the available honey bee plant species contributed 90% of the available honey bee forage (Mensah et al., 2017). It would be interesting to test the extent to which an abundant pollen or nectar source in the landscape reduces the diversity of pollen collected by a colony.

The peak in pollen colour diversity in September was unexpected and does not coincide with the peak flowering time in July. All six colour categories were present from March to October, so the change in diversity was mainly due to changes in equitability. This trend is evident in Fig. 1C, where the numbers of taxa in flower in the three colour categories shown are most similar in August and September and differ much more in April and May. Thus a small, random sample of the plant species available to honey bees in September is likely to contain more of the pollen colour categories than in April. In practice, pollen loads can be divided by eye into many more colours than the six colour categories used here. The problem with this is that a particular number of 'different' colours could consist of rather similar colours with low plant diversity or very different colours with high plant diversity.

Does colour diversity reflect phylogenetic diversity or nutritional diversity? The broad phylogenetic association with pollen colour found here indicates that colour diversity will also broadly reflect phylogenetic diversity. The association between phylogenetic diversity and nutritional diversity is less clear because we have very little knowledge of phylogenetic variation in pollen nutrients or which nutrients might be limiting when the pollen diversity available to honey bees is reduced.

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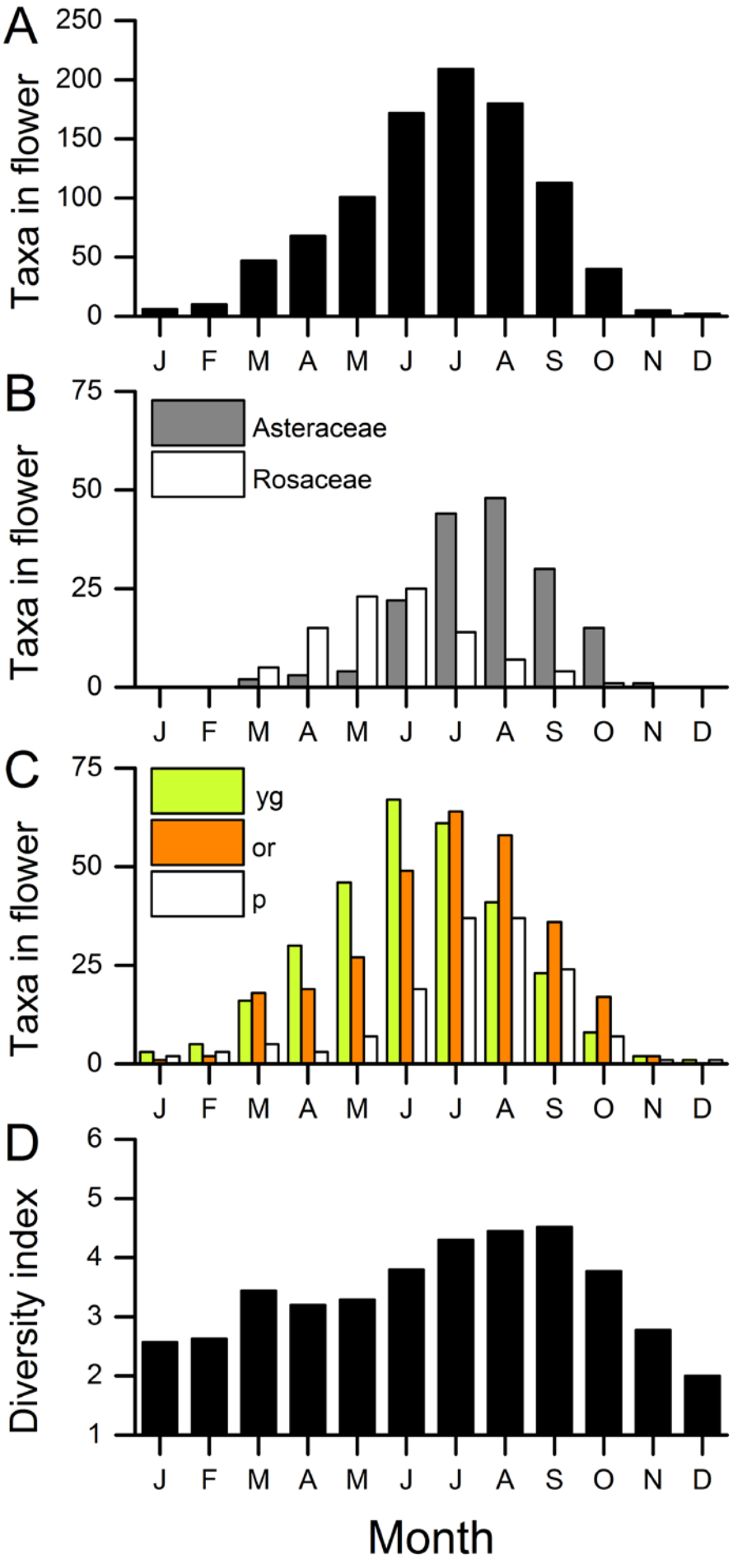
Legend to figure

Figure 1. Changes in the pollen available to honey bees by month through the year for plant taxa from which honey bees collect pollen.

(A) The total number of taxa in flower (*n* = 313). (B) The number of taxa in flower for Asteraceae (*n* = 51) and Rosaceae (*n* = 39). (C) The number of taxa in flower by pollen-load colour category for yellow-green (yg), orange (or) and pale (p) (*n* = 313). (D) Simpson's diversity index for the six colour categories for the taxa in flower in each month. The months are in sequence from January to December, indicated by their initial letter.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Taxa in flower
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Table 1. The numbers of plant taxa in each pollen-load colour category.

Plant group	Pollen-load colour category					
	red	brown	orange	yellow-green	pale	purple-blue
All families	8 (3%)	41 (13%)	**93 (30%)**	**107 (34%)**	47 (15%)	17 (5%)
Asteraceae	0 (0%)	2 (4%)	**36 (71%)**	2 (4%)	**11 (22%)**	0 (0%)
Rosaceae	0 (0%)	**10 (26%)**	5 (13%)	**24 (62%)**	0 (0%)	0 (0%)
Others	8 (4%)	29 (13%)	**52 (23%)**	**81 (36%)**	36 (16%)	17 (8%)

The numbers of plant taxa (with the percentage in brackets) in each pollen-load colour category for all families combined (n = 313), Asteraceae only (n = 51), Rosaceae only (n = 39) and all families other than Asteraceae and Rosaceae (n = 223). The two highest percentages for each plant group are indicated in bold font. See the Results section for chi-squared tests comparing these frequencies between plant groups.